

## SEQUENCE LISTING

Pub D  
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Yabuta, Masayuki  
Suzuki, Yuji

<120> Process for Producing Peptides Using a Helper Peptide

<130> 001560-373

<140> US 09/402,093

<141> 1999-09-29

<150> PCT/JP99/00406

<151> 1999-01-29

<150> JP 10-32272

<151> 1998-01-30

<160> 24

<170> PatentIn version 3.0

<210> 1

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino acid sequence adjacent to a site cleaved by enterokinase.

<400> 1

Asp Asp Asp Lys

1

B1  
<210> 2

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino acid sequence adjacent to a site cleaved by blood  
coagulation Factor Xa.

<400> 2

Ile Glu Gly Arg

1

<210> 3

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

*Sub-D1* <223> Amino acid sequence containing a site cleaved by renin.

<400> 3

Pro Phe His Leu Leu Val Tyr  
1 5

<210> 4

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino acid sequence of helper peptide.

<400> 4

Val Asp Asp Asp Asp Lys  
1 5

<210> 5

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino acid sequence of helper peptide.

<400> 5

Gly Cys His His His His  
1 5

<210> 6

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino acid sequence containing a chemically cleaved site.

<400> 6

Pro Gly Gly Arg Pro Ser Arg His Lys Arg  
1 5 10

<210> 7

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino acid sequence of helper peptide.

<400> 7

His Arg His Lys Arg Ser His His His His

1 5 10

<210> 8  
 <211> 5  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Amino acid sequence containing a site cleaved by Kex2 protease.

<400> 8

Ser Asp His Lys Arg  
 1 5

<210> 9  
 <211> 23  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Amino acid sequence containing a position cleaved by OmpT.

<400> 9

Gln Met His Gly Tyr Asp Ala Glu Leu Arg Leu Tyr Arg Arg His His  
 1 5 10 15

Arg Trp Gly Arg Ser Gly Ser  
 20

<210> 10  
 <211> 20  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Amino acid sequence containing a position cleaved by OmpT.

<400> 10

Gln Met His Gly Tyr Asp Ala Glu Leu Arg Leu Tyr Arg Arg His His  
 1 5 10 15

Gly Ser Gly Ser  
 20

<210> 11  
 <211> 69  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Nucleotide sequence coding for an amino acid sequence containing  
 a site cleaved by OmpT

<220>

B1  
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<221> CDS  
 <222> (1)..(69)

<400> 11

cag atg cat ggt tat gac gcg gag ctc cgg ctg tat cgc cgt cat cac 48  
 Gln Met His Gly Tyr Asp Ala Glu Leu Arg Leu Tyr Arg Arg His His  
 1 5 10 15

cgg tgg ggt cgt tcc gga tcc 69  
 Arg Trp Gly Arg Ser Gly Ser  
 20

<210> 12

<211> 23

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino acid sequence containing a site cleaved by OmpT.

<400> 12

Gln Met His Gly Tyr Asp Ala Glu Leu Arg Leu Tyr Arg Arg His His  
 1 5 10 15

Arg Trp Gly Arg Ser Gly Ser  
 20

<210> 13

<211> 47

<212> DNA

<213> Artificial Sequence

<220>

<223> Nucleotide sequence coding for an amino acid sequence containing  
 a site cleaved by OmpT

<400> 13

tggttatgac gcggagctcc gcctgtatcg ccgtcatcac gggtccg 47

<210> 14

<211> 55

<212> DNA

<213> Artificial Sequence

<220>

<223> Nucleotide sequence coding for an amino acid sequence containing  
 a site cleaved by OmpT

<400> 14

gatccggaac cgtgatgacg gcgatacagg cggagctccg cgtcataacc atgca 55

<210> 15  
 <211> 24  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Primer.

<400> 15  
 gactcagatc ttcctgaggc cgat

24

<210> 16  
 <211> 36  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Primer.

<400> 16  
 aaaggtacct tccgcatgcc gcgatgtcg agaagg

36

<210> 17  
 <211> 20  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Primer.

<400> 17  
 aggccaggaa ccgtaaaaag

20

<210> 18  
 <211> 29  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Primer.

<400> 18  
 aaaatgcac gcacgtaac cgtgcatct

29

<210> 19  
 <211> 627  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Nucleotide sequence coding for a fusion protein comprising GLP-1, helper peptide and beta-galactosidase protective peptide.

<220>  
 <221> CDS  
 <222> (82)..(543)

<400> 19

cccaggcttt acactttatg cttccggctc gtatgttggtg tggaattgtg agcggataac 60

aatttcacac aggaaacagc t atg acc atg att acg gat tca ctg gcc gtc 111  
 Met Thr Met Ile Thr Asp Ser Leu Ala Val  
 1 5 10

gtt tta caa cgt aaa gac tgg gat aac cct gcc gtt acc caa ctt aat 159  
 Val Leu Gln Arg Lys Asp Trp Asp Asn Pro Gly Val Thr Gln Leu Asn  
 15 20 25

cgc ctt gca gca cat ccc cct ttc gcc agc tgg cgt aat agc gac gac 207  
 Arg Leu Ala Ala His Pro Pro Phe Ala Ser Trp Arg Asn Ser Asp Asp  
 30 35 40

gcc cgc acc gat cgc cct tcc caa cag ttg cgc agc ctg aat gcc gaa 255  
 Ala Arg Thr Asp Arg Pro Ser Gln Gln Leu Arg Ser Leu Asn Gly Glu  
 45 50 55

tgg cgc ttt gcc tgg ttt ccg gca cca gaa gcg gtg ccg gca agc ttg 303  
 Trp Arg Phe Ala Trp Phe Pro Ala Pro Glu Ala Val Pro Ala Ser Leu  
 60 65 70

ctg gag tca gat ctt cct gac gcc gat act gtc gtc gtc ccc tca aac 351  
 Leu Glu Ser Asp Leu Pro Asp Ala Asp Thr Val Val Val Pro Ser Asn  
 75 80 85 90

tgg cag atg cac ggt tac gat gcg atg cat ggt tat gac gcg gag ctc 399  
 Trp Gln Met His Gly Tyr Asp Ala Met His Gly Tyr Asp Ala Glu Leu  
 95 100 105

cgc ctg tat cgc cgt cat cac ggt tcc gga tcc cct tct cga cat ccg 447  
 Arg Leu Tyr Arg Arg His His Gly Ser Gly Ser Pro Ser Arg His Pro  
 110 115 120

cgg cat gcg gaa ggt acc ttt acc agc gat gtg agc tcg tat ctg gaa 495  
 Arg His Ala Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu  
 125 130 135

ggt cag gcg gca aaa gaa ttc atc gcg tgg ctg gtg aaa gcc cgt ggt 543  
 Gly Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg Gly  
 140 145 150

taagtcgaca gcccgccctaa tgagcgggct tttttttctc ggaattaatt ctcatgtttg 603

acagcttatc atcgataagc tttta 627

<210> 20  
 <211> 154  
 <212> PRT  
 <213> Artificial Sequence

&lt;220&gt;

&lt;223&gt; Amino acid sequence of a fusion protein comprising GLP-1, helper peptide and beta-galactosidase protective peptide.

&lt;400&gt; 20

Met Thr Met Ile Thr Asp Ser Leu Ala Val Val Leu Gln Arg Lys Asp  
 1 5 10 15

Trp Asp Asn Pro Gly Val Thr Gln Leu Asn Arg Leu Ala Ala His Pro  
 20 25 30

Pro Phe Ala Ser Trp Arg Asn Ser Asp Asp Ala Arg Thr Asp Arg Pro  
 35 40 45

Ser Gln Gln Leu Arg Ser Leu Asn Gly Glu Trp Arg Phe Ala Trp Phe  
 50 55 60

Pro Ala Pro Glu Ala Val Pro Ala Ser Leu Leu Glu Ser Asp Leu Pro  
 65 70 75 80

Asp Ala Asp Thr Val Val Val Pro Ser Asn Trp Gln Met His Gly Tyr  
 85 90 95

Asp Ala Met His Gly Tyr Asp Ala Glu Leu Arg Leu Tyr Arg Arg His  
 100 105 110

His Gly Ser Gly Ser Pro Ser Arg His Pro Arg His Ala Glu Gly Thr  
 115 120 125

Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu  
 130 135 140

Phe Ile Ala Trp Leu Val Lys Gly Arg Gly  
 145 150

&lt;210&gt; 21

&lt;211&gt; 187

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Amino acid sequence of a fusion protein comprising GLP-1, helper peptide and beta-galactosidase protective peptide.

&lt;400&gt; 21

Met Thr Met Ile Thr Asp Ser Leu Ala Val Val Leu Gln Arg Lys Asp  
 1 5 10 15  
 Trp Asp Asn Pro Gly Val Thr Gln Leu Asn Arg Leu Ala Ala His Pro  
 20 25 30  
 Pro Phe Ala Ser Trp Arg Asn Ser Asp Asp Ala Arg Thr Asp Arg Pro  
 35 40 45  
 Ser Gln Gln Leu Arg Ser Leu Asn Gly Glu Trp Arg Phe Ala Trp Phe  
 50 55 60  
 Pro Ala Pro Glu Ala Val Pro Ala Ser Leu Leu Glu Ser Asp Leu Pro  
 65 70 75 80  
 Glu Ala Asp Thr Val Val Val Pro Ser Asn Trp Gln Met His Gly Tyr  
 85 90 95  
 Asp Ala Pro Ile Tyr Thr Asn Val Thr Tyr Pro Ile Thr Val Asn Pro  
 100 105 110  
 Pro Phe Val Pro Thr Glu Pro His His His His His Gly Gly Arg Gln  
 115 120 125  
 Met His Gly Tyr Asp Ala Glu Leu Arg Leu Tyr Arg Arg His His Arg  
 130 135 140  
 Trp Gly Arg Ser Gly Ser Pro Ser Arg His Lys Arg His Ala Glu Gly  
 145 150 155 160  
 Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys  
 165 170 175  
 Glu Phe Ile Ala Trp Leu Val Lys Gly Arg Gly  
 180 185

<210> 22  
 <211> 184  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Amino acid sequence of a fusion protein comprising GLP-1, helper peptide and beta-galactosidase protective peptide.

<400> 22

Met Thr Met Ile Thr Asp Ser Leu Ala Val Val Leu Gln Arg Lys Asp  
 1 5 10 15  
 Trp Asp Asn Pro Gly Val Thr Gln Leu Asn Arg Leu Ala Ala His Pro  
 20 25 30  
 Pro Phe Ala Ser Trp Arg Asn Ser Asp Asp Ala Arg Thr Asp Arg Pro  
 35 40 45  
 Ser Gln Gln Leu Arg Ser Leu Asn Gly Glu Trp Arg Phe Ala Trp Phe



50 55 60

Pro Ala Pro Glu Ala Val Pro Ala Ser Leu Leu Glu Ser Asp Leu Pro  
65 70 75 80

Glu Ala Asp Thr Val Val Val Pro Ser Asn Trp Gln Met His Gly Tyr  
85 90 95

Asp Ala Pro Ile Tyr Thr Asn Val Thr Tyr Pro Ile Thr Val Asn Pro  
100 105 110

Pro Phe Val Pro Thr Glu Pro His His His His His Gly Gly Arg Gln  
115 120 125

Met His Gly Tyr Asp Ala Glu Leu Arg Leu Tyr Arg Arg His His Gly  
130 135 140

Ser Gly Ser Pro Ser Arg His Lys Arg His Ala Glu Gly Thr Phe Thr  
145 150 155 160

Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu Phe Ile  
165 170 175

Ala Trp Leu Val Lys Gly Arg Gly  
180

<210> 23  
<211> 184  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Amino acid sequence of a fusion protein comprising GLP-1, helper peptide and beta-galactosidase protective peptide.

<400> 23

Met Thr Met Ile Thr Asp Ser Leu Ala Val Val Leu Gln Arg Lys Asp  
1 5 10 15

Trp Asp Asn Pro Gly Val Thr Gln Leu Asn Arg Leu Ala Ala His Pro  
20 25 30

Pro Phe Ala Ser Trp Arg Asn Ser Asp Asp Ala Arg Thr Asp Arg Pro  
35 40 45

Ser Gln Gln Leu Arg Ser Leu Asn Gly Glu Trp Arg Phe Ala Trp Phe  
50 55 60

Pro Ala Pro Glu Ala Val Pro Ala Ser Leu Leu Glu Ser Asp Leu Pro  
65 70 75 80

Glu Ala Asp Thr Val Val Val Pro Ser Asn Trp Gln Met His Gly Tyr  
85 90 95

Asp Ala Pro Ile Tyr Thr Asn Val Thr Tyr Pro Ile Thr Val Asn Pro  
100 105 110

Pro Phe Val Pro Thr Glu Pro His His His His His Gly Gly Arg Gln  
 115 120 125

Met His Gly Tyr Asp Ala Glu Leu Arg Leu Tyr Arg Arg His His Gly  
 130 135 140

Ser Gly Ser Pro Ser Arg His Pro Arg His Ala Glu Gly Thr Phe Thr  
 145 150 155 160

Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu Phe Ile  
 165 170 175

Ala Trp Leu Val Lys Gly Arg Gly  
 180

<210> 24

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino acid sequence containing a site cleaved by Kex2 Protease.

<400> 24

Ser Cys His Lys Arg  
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